Toxicology and Applied Pharmacology 348 (2018) 117–122

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

High impact short article

Silicosis decreases bone mineral density in rats

Zhang Hui[a,1], Xu Dingjie[b,1], Yuan Yuan[c,1], Wei Zhongqiu[a], Mao Na[a], Bei Mingjian[d], Gou Yu[d], Liu Guangyuan[d], Gao Xuemin[a], Li Shifeng[a], Geng Yucong[a], Yang Fang[a], Ross Summer[d,**], Xu Hong[a,*]

[a] Medical Research Center, North China University of Science and Technology, Tangshan, China
[b] College of Traditional Chinese Medicine, North China University of Science and Technology, Tangshan, China
[c] Pathology Department, Beijing Tiantan Hospital, Beijing, China
[d] Center for Translational Medicine, Jane and Leonard Korman Respiratory Institute, Thomas Jefferson University, Philadelphia, PA, United States

ARTICLE INFO

Keywords:
Silicosis
Osteoporosis
Bone mineral density
Osteoclast

ABSTRACT

Silicosis is the most common occupational lung disease in China, and is associated with a variety of complications, many of which are poorly understood. For example, recent data indicate that silicosis associates with the development of osteopenia, and in some cases this bone loss is severe, meeting criteria for osteoporosis. Although many factors are likely to contribute to this relationship, including a sedentary lifestyle in patients with advanced silicotic lung disease, we hypothesized that silica might directly reduce bone mineral density. In the present study, six Wistar rats were exposed to silica for 24 weeks in order to induce pulmonary silicosis and examine the relationship to bone mineral density. As expected, all rats exposed to silica developed severe pulmonary fibrosis, as manifested by the formation of innumerable silicotic nodules and the deposition of large amounts of interstitial collagen. Moreover, micro-CT results showed that bone mineral density (BMD) was also significantly reduced in rats exposed to silica when compared control animals and this associated with a modest reduction in serum calcium and 25-hydroxyvitamin D levels. In addition, we found that decreased BMD was also linked to increased osteoclast activity as well as fibrosis-like changes, and to the deposition of silica within bone marrow. In summary, our findings support the hypothesis that silicosis reduces bone mineral density and provide support for ongoing investigations into the mechanisms causing osteopenia in silicosis patients.

1. Introduction

Silicosis is an irreversible, often fatal, fibrotic pulmonary disease that develops after prolonged inhalation of crystalline silicon dioxide or silica; it is one of the most important occupational diseases worldwide (Leung et al., 2012). China has the most patients with silicosis, with approximately 20,000 new cases diagnosed annually. Silicosis is also known to develop in association with several other disorders, including tuberculosis and various other infections, chronic obstructive pulmonary disease, lung cancer, autoimmune diseases like scleroderma and rheumatoid arthritis as well as kidney disease (Leung et al., 2012). Recently, it has been reported by several groups that osteoporosis is also more common in patients with silicosis (Yildizgören et al., 2014) as judged by the reduction in bone mineral density (BMD) in patients with silicosis when compared to age-matched controls. Interestingly, it has also been shown that the amount of bone loss correlates with the severity of lung disease in humans and horses, suggesting that the intensity of silica exposure might be an important trigger of bone remodelling (Li et al., 2012; Yıldızgören et al., 2016). Moreover, humans with silicosis also develop calcium deposition outside the lung, which could be interpreted as evidence of systemic calcium dysregulation (Arens et al., 2011). With this evidence in mind, our hypothesis is that silicosis reduces bone mineral density by directly promoting bone resorption and remodelling.

2. Materials and methods

2.1. Silicosis disease model

Male Wistar rats (aged 3 weeks) were purchased from Vital River

* Correspondence to: X. Hong, Medical Research Center, North China University of Science and Technology, No. 21 Bohai Road, Caofeidian Eco-city, Tangshan, Hebei 063000, China.
** Correspondence to: R. Summer, Pulmonary & Critical Care Medicine, Jane and Leonard Korman Respiratory Institute, Thomas Jefferson University, Philadelphia, PA 19107, United States.
E-mail addresses: ross.summer@jefferson.edu (R. Summer), Hong.Xu2@jefferson.edu (X. Hong).
[1] Co-first author.

https://doi.org/10.1016/j.taap.2018.04.023
Received 26 February 2018; Received in revised form 17 April 2018; Accepted 18 April 2018
Available online 19 April 2018
0041-008X/

Laboratory Animal Technology Co. Ltd. (SCXY 2009–0004, Beijing, China). The environmental conditions were 25 °C ± 1 °C, 55% ± 10% humidity, and 12:12 light: dark cycle. A HOPE MED 8050 exposure control apparatus (HOPE Industry and Trade Co. Ltd., Tianjin, China) was used to create the silicosis model (Fig. S1) with the following parameters: exposure chamber volume 0.3 m^3, cabinet temperature 20–25 °C, humidity 70–75%, pressure −50 Pa to +50 Pa, oxygen concentration 20%, flow rate of SiO_2 (s5631, Sigma-Aldrich, USA) 3.0–3.5 ml/min, and dust mass concentration in the cabinet 2000 mg/m^3; each animal was instilled for 3 h per day (Dai et al., 2016). Rats were exposed to silica for 24 w ($n = 6$). Rats in the control group (n = 6) were placed in the exposure control apparatus without silica for 3 h/day under the same conditions. All animal experiments were performed in accordance with the regulations set by the Committee on the Ethics of North China University of Science and Technology.

2.2. Morphological observation

The lung, heart, kidney, spleen, femur, and tibia were fixed in 4% neutral formalin solution for 48 h. The bone was decalcified with 15% EDTA-Na_2 (pH 7.25) at 4 °C for 6 weeks (Liu et al., 2017). The samples were sequentially dehydrated, embedded in paraffin, and cut into 4 μm (lung, heart, kidney, and spleen) or 8 μm (bone) sections. The pathologic and morphologic characteristics of the tissues were then observed by haematoxylin and eosin (H&E, BA4025, Baso Diagnostics Inc., Zhuhai, China), Masson's (BA4079, Baso Diagnostics Inc., Zhuhai, China), and Van Gieson's (VG, BA4084, Baso Diagnostics Inc., Zhuhai, China) staining. Silica was observed using a polarized light microscope. The fibrotic area was measured using cellSens Dimension software (Olympus Corporation, Tokyo, Japan).

2.3. Subchondral bone microstructure measurements

The proximal tibiae and distal femur were scanned using a micro-CT system (ZKKS-Sharp-MCT, Guangzhou, China) to quantify the micro-architecture of subchondral trabecular bone, and the region of interest (ROI). The ROI was determined based on the area in which fibrosis was most apparent, and was defined as the epiphyseal cancellous bone region 0.5 mm from the subchondral plate to growth plate of tibiae or femur, with voxel size of 2 mm (Liu et al., 2017). The energy and intensity were 40 kVp and 250 mA, respectively. The following morphometric parameters were calculated using software developed for the machine to describe the bone mass and structure: bone mineral density (BMD), present bone volume, trabecular thickness, trabecular separation, trabecular number, trabecular pattern factor, and structure model index (SMI).

2.4. Immunohistochemical(IHC) assessment

Paraffin sections were deparaffinized and rehydrated, and endogenous peroxidases were quenched with 3% H_2O_2 for 15 min. The samples were then incubated with primary antibodies against transforming growth factor (TGF)-β_1(AF1027, Affinity Biologicals, Ganada), vimentin (ab92547, Abcam, Cambridge, MA, USA), Osterix (ARG65886, arigo biolaoratories, Taiwan, China), α-smooth muscle actin (α-SMA, ab3275, Eptomics, Mitten, CA, USA), CD34 (ab81289, Abcam, Cambridge, MA, USA), and CD68 (ab955, Abcam, Cambridge, MA, USA) overnight at 4 °C, followed by incubation with secondary antibodies (PV-6000, Beijing Zhongshan Jinqiao Biotechnology Co. Ltd., Beijing, China) at 37 °C for 20 min. Immunoreactivity was visualized using DAB (ZLI-9018, Beijing Zhongshan Jinqiao Biotechnology Co. Ltd., Beijing, China). Brown staining was considered a positive result.

The number of positive cells in TGF-β_1(osteoclast (Kasagi and Chen, 2013)), vimentin(osteoclast(Faloni et al., 2012; Akisaka et al., 2008)), and Osterix (osteoblast precursor cells (Ma and Xu, 2018)) was counted by IPP 6.0 image analysis software (Media Cybernetics, USA), 3–5 fields of view were selected on each section and photographed.

2.5. Serological test

The serum levels of calcium, phosphate, 25-hydroxyviamin D and parathyroid hormone (PTH) were measure by calcium assay kit (C004-2, Nanjing Jiancheng Bioengineering Institute, China), phosphate assay kit (C006, Nanjing Jiancheng Bioengineering Institute, China), 25 hydroxyvitamin D assay kit (H191, Nanjing Jiancheng Bioengineering Institute, China), and rat PTH Enzyme-linked immunosorbent assay (ELISA) (MB-2075A, Jiangsu Mei Biao Biological Technology Co., Ltd., China), respectively. All the tests were followed the suggested manufacture's protocol.

2.6. Statistical analysis

Values are expressed as means ± SD. Independent-samples *t*-tests were used for comparison between two groups. Group differences with *p*-values < 0.05 were considered statistically significant.

3. Results

3.1. BMD is decreased in rats exposed to silica

We have demonstrated that prolonged exposure to silica using a HOPE MED8050 apparatus closely approximates the development of silicosis in humans (Dai et al., 2016). Consistent with this, we found that rats exposed to silica for 24 w developed severe pulmonary fibrosis as judged by the formation of innumerable silicosis nodules and the deposition of extensive amounts of collagen (Fig. 1 A and B). To assess BMD in these rats we measured various parameters in the tibia and femur using micro-CT. As shown in Fig. 1C, D, and E, we detected a significant decrease in BMD in rats exposed to silica when compared to controls. Moreover, this was associated with other changes in subchondral bone micro-architecture including a reduction in percent bone volume, trabecular separation, trabecular number, and structure model index and only trabecular thickness and trabecular pattern were unaffected by silica exposure (Table 1).

3.2. Serum calcium and 25-hydroxyviamin D levels are reduced in silica-exposed rats

Standard assays for evaluating the etiology of decreased bone mineral density in humans include measuring serum levels of calcium, phosphate, 25-hydroxyviamin D and PTH as well as performing liver and kidney function tests (Sheu and Diamond, 2016). We found that serum calcium and 25-hydroxyviamin D levels were reduced in rats exposed to silica but phosphate and PTH levels were not significantly affected by silica exposure (Fig. 2). Although liver and kidney function tests were not performed we observed mild degenerative changes in the proximal convoluted tubules of silica exposed kidneys, suggesting that calcium resorption or excretion by the kidney could be altered (Fig. S2). Among the other organs evaluated, including heart, liver and spleen we did not observe morphological differences between control and silica-exposed rats.

3.3. Silica induces osteoclast activation

Bone modelling describes the process whereby bone structure is reshaped by the action of osteoblasts and osteoclasts (Kasagi and Chen, 2013). Evidence of bone remodelling after exposure to silica was suggested by increased fibrosis-like changes in the bone marrow as well as enhanced staining for vimentin, a marker of osteoclast activation. Further, we found that cells staining for vimentin (Fig. 3A and B) also appeared to express high levels of TGF-β1 (Fig. 3C and D), suggesting